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Plasma Membrane Dehydrogenases in Rat Brain Synaptic Membranes. Multiplicity and Subunit Composition

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Abstract

Plasma membrane redox enzymes have been investigated in synaptic membranes from rat brain nerve terminals. UV-Vis spectra of intact synaptic plasma membranes are presented and the presence of a *b*-type cytochrome, detectable at 77°K and sensitive to NADH or NADPH, is shown. The molecular characterization of rat synaptic NADH-dehydrogenases was further performed on solubilized enzymes using a recently developed nondissociating polyacrylamide gel electrophoresis technique. Synaptic plasma membranes were solubilized with 1% sodium cholate or Triton X-114 and centrifuged. The supernatant retained over 60% of the NADH-dehydrogenase activity, tested with either DCIP[2] or ferricyanide as substrates, together with NADH. Both enzyme activities were insensitive toward rotenone. This extraction procedure also solubilized about 50% of the proteins. When submitted to polyacrylamide gel electrophoresis under nondenaturing conditions and stained for NADH-dehydrogenase activity, five bands of different mobilities were detected. The multiple NADH-dehydrogenases of synaptic plasma membranes were investigated by means of band excision and the five excised bands each submitted to amino acid analysis and to 2-D electrophoresis. The subunit composition of each band was then deduced, together with the molecular weight and p*I* of each respective subunit. NADH-dehydrogenases have also been purified by means of FPLC on Mono-P (chromatofocusing) followed by gel filtration on Superose 12. NADH-Dehydrogenase IV and V could be purified in their active forms by this approach.

Key Words: Plasma membrane NADH dehydrogenases; neurotransmitters.

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[2]Abbreviations: DCIP, dichlorophenol-indophenol; FPLC, fast protein liquid chromatography.

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Introduction

Plasma membrane dehydrogenases present a considerable challenge in terms of protein characterization because of their low tissue abundance and the complexity of the processes involved (Crane *et al.*, 1988). The real function of transplasma-membrane NADH-dehydrogenases has not been established so far. In synaptic plasma membranes this redox activity is linked to post-receptor signal transduction phenomena (Treichler and Dreyer, 1986). However, in terms of substrate oxidation, synaptic plasma membranes contain several distinct NADH-dehydrogenases, a possibility which has long been suggested on the basis of inhibitor sensitivity and substrate affinity (Dreyer *et al.*, 1989). The heterogeneity of synaptic plasma membrane preparations makes any assessment about the specific function of each NADH-dehydrogenase difficult. Therefore a methodology for separating the diverse NADH-dehydrogenase activities is desirable.

In this paper, we present additional evidence for the presence of redox components in intact synaptic membranes by means of UV-Vis spectroscopy. Furthermore, we have used a recently developed Triton X-100-containing nondenaturing gel electrophoresis method in order to characterize synaptic plasma membrane NADH-dehydrogenases. This technique, which is particularly suitable for membrane compounds (Kuonen *et al.*, 1986), has permitted us to identify multiple NADH dehydrogenase activities, which have been further analyzed for polypeptide composition by denaturing-gel electrophoresis and isoelectric focusing.

Material and Methods

Materials

Triton X-100 and X-114 were purchased from Merck, Zwittergent ZW-14 from Calbiochem, and Ficoll from Pharmacia. Radiochemicals were from Radiochemical Centre Amersham. All other reagents were from Sigma or Boehringer.

Preparation of Synaptic Membranes

Adult Sprague-Dawley rats (180–220 g) were used throughout this study. The rats were decapitated and the whole brains were soaked in 10 volumes ice-cold 0.32 M sucrose solution. Synaptic membranes were prepared as described by Cotman and Mattews (1971). The brains were homogenized at 20% w/v in 0.32 M sucrose and diluted to 7% (w/v) for centrifugation at 1100 $\times$ g for 5 min. The supernatant was centrifuged at

17,000 × *g* for 10 min, and the crude mitochondrial fraction was suspended in 10% sucrose and applied to a two-step discontinuous Ficoll–sucrose gradient (13% (w/v) and 7.5% (w/v) Ficoll, respectively, in 0.32 M sucrose. The synaptosomal fraction that appeared at the interface upon centrifugation for 90 min at 65,000 × *g* was diluted with four volumes of 10% (w/v) sucrose and pelleted at 30,000 × *g* for 30 min. Osmotic shock was carried out as in Cotman and Mattews (1971) in five volumes of Tris-HCl 10 mM, pH 7.4, for 45 min. The fraction was concentrated and applied to a discontinuous gradient of 5-ml layers of 25, 32.5, 35, and 38% sucrose (w/v), respectively. Centrifugation was carried out for 1.5 h at 65,000 × *g*. The fraction at the interface of 25–32 5% sucrose was collected and contained the synaptic membrane preparations, according to Cotman and Mattews (1971). Alternately the purification procedure outlined by Lopez-Perez *et al.* (1981) using the phase partition technique in polyethylene glycol 4,000–Dextran T500 for the purification of synaptosomes was used.

Extraction Procedure

Treatment of the synaptic membranes with sodium cholate was performed in 50 mM Tris-HCl, pH 7.4, containing 0.5% sodium cholate. The membranes were gently homogenized by hand homogenization in a Potter–Elvejem homogenizer for 1 min followed by a 45 min incubation at room temperature. The suspension (2–3 mg of protein per ml) was then centrifuged for 30 min at 100,000 × *g* at 4°C. The final pellet was resuspended in 50 mM Tris-HCl, pH 7.4.

Plasma Membrane NADH-dehydrogenase Activity

NADH-dehydrogenase activity in synaptic membrane preparations was determined spectrophotometrically according to the outlines of Crane and Loew (1976) or Goldenberg *et al.* (1979) in 0.1 M buffer at pH 8.0. Buffers included potassium phosphate or Hepes. All measurements were performed on a UV-3000 spectrophotometer from Shimadzu, in 1-cm standard quartz cuvettes.

NADH-indophenol reductase (DCIP reductase) was determined with 0.05 mM NADH and 0.05 mM 2,6-dichloroindophenol added to 1.0 ml of the incubation mixture (0.1 M potassium phosphate buffer, pH 8.0) to start the reaction. Absorbancy was recorded at 600 nm.

For ferricyanide reductase, the reaction mixture consisted of 0.1 M potassium phosphate, pH 7.0, 0.3 mM $K_3(Fe(CN)_6)$, 1.5 mM NADH, and enzyme (50–200 μg of protein). Ferricyanide reduction was recorded at 420 nm ($\varepsilon = 1\,mM^{-1}$).

Gel Electrophoresis

Nondenaturing-gel electrophoresis was performed at 4°C in 8% (w/v) polyacrylamide gels containing 1% (w/v) Triton X-100 and 100 mM Tris-glycine buffer, pH 9.0. Samples were applied in a single continuous band across the gel. Staining of gels for NADH-dehydrogenase activity was performed by using 1 mM NADH in 50 mM phosphate-buffer, pH 7.4, which contained 1 mg of nitroblue tetrazolium/ml. The staining was performed overnight at 4°C. Band excision of the five bands was immediately performed the next morning. The bands were then incubated in 2 ml of 50 mM phosphate buffer that contained 1% Triton X-100 and sonicated six times for 1 min (Braun Labsonic 1510) at 100 W in the cold. The extracts were then concentrated to ca. 20–40 μl using Centricon tubes (Amicon) and stored at −80°C until used. Samples of 1–2 μl were used for 2-D gel electrophoresis using a PHAST-Pharmacia apparatus and silver stained.

Other Analysis

Amino acid analysis of the NADH-dehydrogenases was performed on the enzymes purified by band excision. Samples were hydrolyzed in sealed evacuated tubes for 24 h at 100°C in 6 M Suprapur HCl (Merck) containing 2 mg/ml of phenol and an appropriate amount of norleucine as internal standard. The hydrolysates were analyzed on a Chromakon 500 amino acid analyzer from Kontron, interfaced with an Anacomp 220 microcomputer. Protein was determined as described by Lowry *et al.* (1951). Enzyme assays for marker enzymes included cytochrome oxydase (EC 1.9.3.1) performed according to Duncan and Mackler (1966); (Na^+-K^+)-activated, ouabain-sensitive ATPase (EC 3.6.1.4) assayed as described in Cotman *et al.* (1971); acid phosphatase (EC 3.1.3.2) assayed according to Lowry *et al.* (1954); alkaline phosphatase (EC 3.1.3.1), 5'-nucleotidase (EC 3.1.3.5) and nucleoside diposphatase (EC 3.6.1.6) were all assayed as in Emmelot *et al.* (1964). For β-*N*-acetylglucosaminidase (EC 3.2.1.30) and acetylcholinesterase (EC 3.1.1.7), the outlines of Sellinger *et al.* (1960) and Ellmann (1961), respectively, were followed. Succinic acid dehydrogenase (EC 1.3.99.1) and NADH-cytochrome *c* reductase (EC 1.6.2.1) were tested as in Singer and Gutman (1971) and Dallner *et al.* (1966), respectively.

Instrumentation

Light absortion spectra at 77°C were recorded on a UV-3000 spectrophotometer from Shimadzu in 50% glycerol containing 50 mM Tris-HCl, pH 7.4. Reduction proceeded by addition of either 5 μl NADH or 50 mM NADPH or a few crystals of solid dithionite to the thawed sample.

Results and Discussion

Purified synaptic plasma membranes prepared according to the established procedure used in this study are normally devoid of enzyme activity from mitochondrial or microsomal origin (in agreement with Cotman and Mattews 1971), but consist of a mixture of plasma membranes from different brain cell types. The purity of the membrane preparations was routinely checked on each batch (Table I); in particular, contaminations from mitochondrial and microsomal origin were carefully tested. On some occasions, e.g., when seeking a thorough exclusion of contaminating mitochondria, minor modifications in the procedure were introduced, mainly by repeating the final centrifugation step in 13% Ficoll/0.32 M sucrose under identical conditions. The data presented in this paper were all taken from plasma membranes of particularly high purity.

UV-Vis spectroscopy in liquid nitrogen was performed on such preparations free of intracellular redox activities (Fig. 1). The membranes were incubated in 50 mM Tris-HCl that contained 50% glycerol (final concentration) and microcrystallized by multiple freezing and thawing. At 77°C, air-oxidized samples exhibited a well-resolved spectrum with peaks at 572, 558, 538, and 417 nm and shoulders at 544 and 542 nm, together with a broad shoulder around 440–470 nm. Upon thawing and reduction with excess NADH, the peaks at 558 and 538 nm disappeared, while the other peaks remained little affected. Similar effects were also observed when NADPH was

Table I. Marker Enzyme Activities in Rat Brain Synaptic Membrane Fractions[a]

Enzyme activity	Cell fraction		
	Endoplasmic recticulum	Mitochondrial membrane	Synaptic membrane
Na^+-K^+-ATP-ase	3.0	57.0	38.0
Alkaline phosphatase	5.0	25.0	11.0
5'-Nucleotidase	15.0	22.0	35.0
Acetylcholinesterase	8.0	40.0	40.0
Acid phosphatase	7.0	72.0	5.0
β-N-Acetylglucosaminidase	0.0	53.0	0.0
Cytochrome oxidase	0.0	95.0	0.0
Nucleoside diphosphatase	38.0	56.0	0.0
Succinate dehydrogenase	30.0	65.0	0.0
Antimycin-insensitive NADH-cytochrome *c* reductase	5.0	40.0	7.0

[a] Synaptic membrane fractions were prepared by differential and density gradient centrifugation according to Cottman and Mattews, 1971. The brains were homogenized at 20% w/v in 0.32 M sucrose, diluted to 70% w/v for centrifugation, and processed as described in the Material and Methods section. Activities are expressed as relative specific activities (percent total activity/percent total protein).

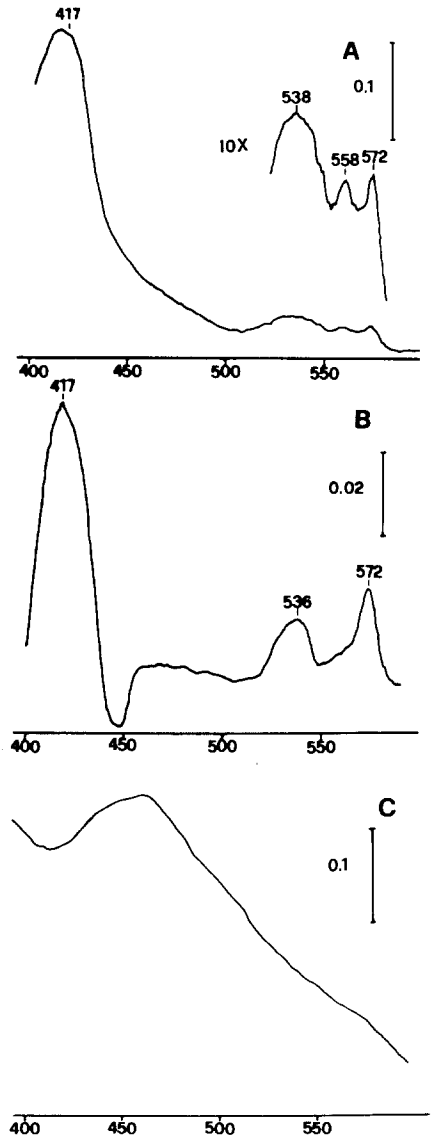

Fig. 1. UV-Vis spectra at 77°K of synaptic plasma membranes. The synaptic membranes were incubated in 50 mM Tris-HCl, pH 7.4, containing 50% glycerol and 0.5% Lubrol. (A) Oxidized spectrum without addition; (B) synaptic plasma membranes reduced with 5 mM NADH; (C) synaptic plasma membranes reduced with solid sodium dithionite. All measurements were performed at 77°K in liquid nitrogen.

used as reductant. Upon further reduction with solid sodium dithionite in excess, the heme spectrum vanished completely, but a flavin-type spectrum remained together with additional aborption in the 500–630 nm region. No attempts were made so far to determine the redox potentials of the species observed. Reduced pyridine hemochromogen yielded a spectrum identical to the NADH or NADPH reduced species, an indication of the presence of a *b*-type cytochrome in the synaptic plasma membrane. However, the air-oxidized spectrum speaks in favor of several redox species being present.

Earlier data have shown the presence of NADH-dehydrogenase in synaptic plasma membranes (Treichler and Dreyer, 1986). In untreated membrane preparations of high purity such as those employed in this study, the NADH-dehydrogenase activities are rotenone insensitive when both ferricyanide of DCIP were used as substrate, and no test for rotenone sensitivity was performed on the purified enzymes described below. The presence of various NADH-dehydrogenases in the synaptic plasma membranes used in our studies was suggested from the differential sensitivity toward neuroactive agents and neurotransmitters when various oxidants were used as substrates (Treichler and Dreyer, 1986; Dreyer *et al.*, 1989). The multiplicity of NADH-dehydrogenases in synaptic plasma membranes was investigated by comparing staining patterns of extracts in a nondenaturing-gel system. Sodium cholate extracts have been submitted to polyacrylamide gel electrophoresis under nondenaturating conditions on 8% (w/v) continuous polyacrylamide gels containing 0.1% Triton X-100. Upon staining for specific NADH-dehydrogenase activity, five bands of different mobility were clearly and reproducibly detected, as shown in Fig 2. The NADH-dehydrogenase activities thus detected corresponded to lipoprotein complexes of high molecular masses. Protein staining by the method of Sammons *et al.* (1981), routinely performed on one portion of the very same gels, only allowed for the detection of bands I and II, the remaining ones being beyond the detection limits of the silver staining method. Each NADH-dehydrogenase staining band (designated as enzyme I–V, respectively) was carefully excised and concentrated.

Amino acid analysis was performed on the purified enzymes obtained by band excision, and the data are shown in Table II. The five enzymes exhibited approximately 1154, 1246, 974, 682, and 1041 amino acid residues. NADH-dehydrogenases appear particularly rich in glycine (with ratios of 25, 38, 45, 28, and 32% for enzymes I–V, respectively), histidine (28, 34, 15, 4, and 23%), and other basic amino acids residues. Enzymes I, II, and III contain low amounts of cysteine and proline residues (less than 1%), whereas by contrast, enzymes IV and V are devoid of proline but are very rich in cysteine residues, which account for 27 and 35%, respectively, of the total amino acid residues. Dehydrogenase V is devoid of alanine and tyrosine, in contrast to the other four enzymes.

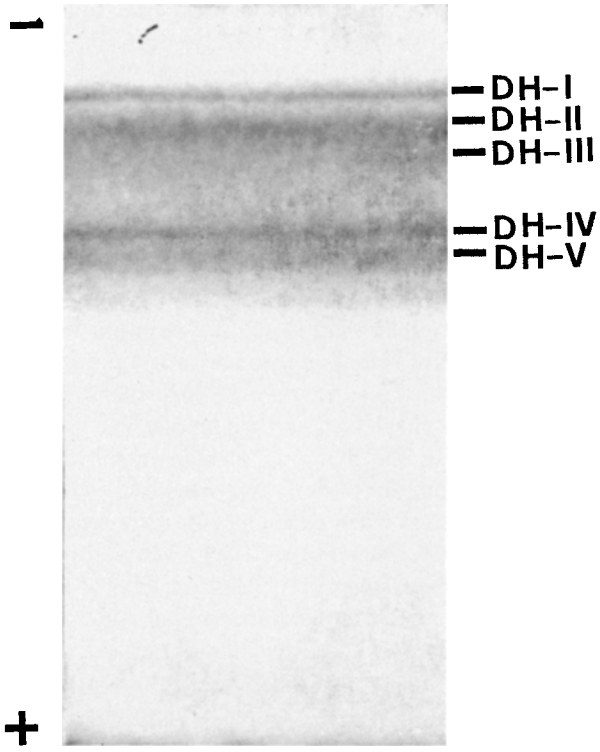

Fig. 2. Nondissociating polyacrylamide-gel electrophoresis of solubilized synaptic plasma membrane. Purified synaptic membranes were solubilized as described in the Materials and Methods section and the extract submitted to electrophoresis. The gel was stained overnight for NADH-dehydrogenase activity with 10 mM NADH and tetra-nitroblue tetrazolium in 50 mM phosphate buffer, pH 7.4. DH-I to DH-V designate five NADH-dehydrogenases.

On the basis of the amino acid composition, a minimal molecular mass of 134.2, 141.1, 68.6, 52.8, and 78.1 kDa for NADH-dehydrogenases I–V, respectively, was calculated (Table III). An estimation of the molecular masses of the native NADH-dehydrogenase complexes was also made by means of chromatography on calibrated columns of Superose 12 (Pharmacia), in order to compare them with the data obtained from amino acid analysis. However, the five enzymes obtained by band excision migrated as large lipoprotein complexes. Although single peaks were obtained for each excised band under these conditions, calculated molecular masses of ca. 240,000–280,000 kDa were obtained.

Each excised NADH-dehydrogenase staining band was further submitted to 2-D electrophoresis under denaturating conditions, for estimation of the subunit composition of these protein complexes (Fig. 3 and Table III).

Table II. Amino Acid Composition of Synaptic Membrane Redox Enzymes, Band I–V[a]

Amino acid	Band I	Band II	Band III	Band IV	Band V
Aspartic acid	37	14	14	7	9
Threonine	26	11	8	3	3
Serine	55	25	24	9	17
Glutamic acid	36	11	14	9	14
Proline	6	2	3	—	—
Glycine	294	475	424	194	334
Alanine	207	166	269	208	—
Cysteine[b]	8	3	5	182	375
Valine	22	9	8	5	3
Methionine	7	2	11	4	6
Isoleucine	15	6	5	3	3
Leucine	40	16	14	8	12
Tyrosine	3	1	3	3	—
Phenylalanine	13	6	3	4	3
Lysine	52	18	22	12	17
Histidine	317	475	139	30	240
Arginine	16	6	5	1	5
Total	1154	1246	973	682	1041

[a]The values were calculated from the amino acid compositions measured after 24 h hydrolysis.
[b]As half-cystine.

The peptides were routinely designed according to their respective intensity. For NADH-dehydrogenase I, only two major bands could be detected, corresponding to polypeptides Ia and Ib, with molecular masses of 43 kDa and p*I* of 6.7 and 6.9, respectively. 2-D electrophoresis of NADH-dehydrogenase II exhibited three visible bands with masses of 68, 92, and 110 kDa, respectively. Peptide IIa exhibited a p*I* of ca. 6.4, while peptides IIb and IIc were more acidic with p*I*'s of 4.9 each. NADH-dehydrogenase III resolved into four polypeptides of masses 90, 46, 75, and 94 kDa, respectively, with corresponding p*I* of 6.3 and 5.4 for peptides IIIa and IIIc, whereas the more acidic peptides IIIb and IIId gave p*I*'s of 5.1 each. Similarly, NADH-dehydrogenase IV also yielded four polypeptides, with molecular masses of ca. 55, 60, 65, and 68 kDa and p*I*'s of ca. 6.4, 5.5 for peptides IVc and IVd, and 8.0 for peptides IVa and IVb, respectively. Finally, 2-D electrophoresis of NADH-dehydrogenase V gave five visible polypeptides with masses of 34, 120, 60, 64, and 108 kDa, respectively, which exhibited p*I*'s of ca. 7.7, 7.6, 6.4 for peptides Va–Vc and 5.1 for peptides Vd and Ve.

Band excision of PAGE-purified enzymes is a rapid method, yielding purified enzymes in a reproducible way and allowing for easy separation of the multiple species present in the membrane preparations. Synaptic plasma membrane preparations are indeed heterogeneous, made of pre-and post-synaptic plasma membrane, besides plasma membranes from glial cells and minor contaminants from intracellular origin. Therefore the presence of

Table III. Apparent Molecular Masses of Synaptic Plasma Membrane Redox Enzymes[a]

Enzymes from gel excision	Minimal MW[b]	
NADH-DH I	134,232	
NADH-DH II	141,106	
NADH-DH III	93,326	
NADH-DH IV	68,764	
NADH-DH V	120,260	
Enzymes from FPLC	MW[c]	
NADH-DH A	62,000	
NADH-DH B	65,000	
Subunits	MW[c]	p*I*[d]
NADH-DH I		
Ia	43,000	6.7
Ib	43,000	6.9
NADH-DH II		
IIa	68,000	6.4
IIb	92,000	4.9
IIc	110,000	4.9
NADH-DH III		
IIIa	90,000	6.3
IIIb	46,000	5.1
IIIc	75,000	5.4
IIId	94,000	5.1
NADH-DH IV		
IVa	55,000	8.0
IVb	60,000	8.0
IVc	65,000	6.4
IVd	68,000	5.5
NADH-DH V		
Va	34,000	7.7
Vb	120,000	7.6
Vc	60,000	6.4
Vd	64,000	5.1
Ve	108,000	5.1

[a] Molecular masses (in daltons) were calculated either from the amino acid composition, where available (see Table II), or from gel electrophoresis under denaturating conditions (SDS-PAGE). p*I* were taken from 2-D gel electrophoresis.
[b] Calculated from amino acid composition.
[c] From SDS-PAGE.
[d] From 2-D IEF electrophoresis.

multiple NADH-dehydrogenases is of no surprise, and the real origin and very function of each of the five enzymes described remains to be established. In addition, NADH-dehydrogenases obtained by band excision are no longer active, since the enzymes have lost their activities by the overnight staining process and because of irreversible sticking of dye by-products to the proteins. For these reasons, attempts were also made to purify the enzymes by

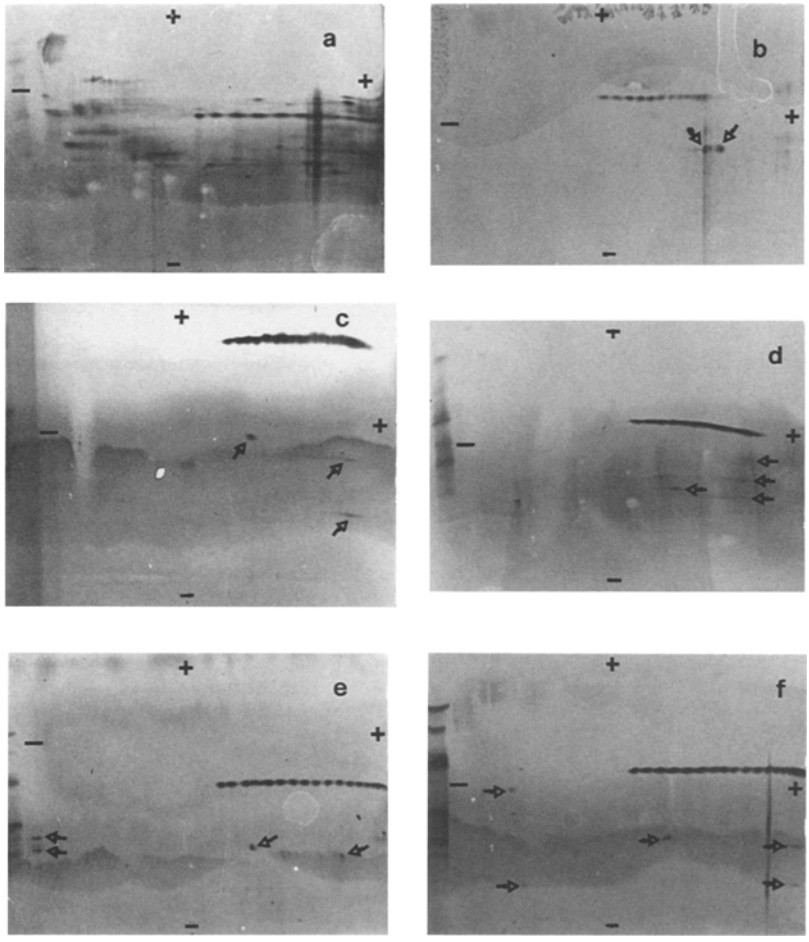

Fig. 3 Two-dimensional sodium dodecyl sulfate polyacrylamide gel of excised bands I–V. Bands I–V from nondissociating polyacrylamide gels were excised carefully and the protein extracted with 1% Triton X-100 in 50 mM Tris by sonication in ice for 6 × 1 min and concentrated to 5–10 μl (Centricon tubes, Amicon), 2-D electrophoresis was then performed by horizontal micro two-dimensional electrophoresis using Phast System (Pharmacia) with immobilized pH gradients in the first dimension (horizontal in the figure), followed by second-dimensional SDS-PAGE (vertical in the figure). The technique follows essentially the outlines of the manufacturer, modified from Görg *et al.* (1988), with Carbamalyte (Pharmacia) as internal protein standards and pH gradient monitors in the first dimension and standard proteins (Pharmacia calibration kit) in the second dimension (SDS-PAGE). The gels were silver-stained according to the Sammons *et al.* (1981) technique. (a) Extract; (b) NADH-dehydrogenase I; (c) NADH-dehydrogenase II; (d) NADH-dehydrogenase III; (e) NADH-dehydrogenase IV; (f) NADH-dehydrogenase V. The arrows on the figure indicate the position of the subunit detected upon silver staining.

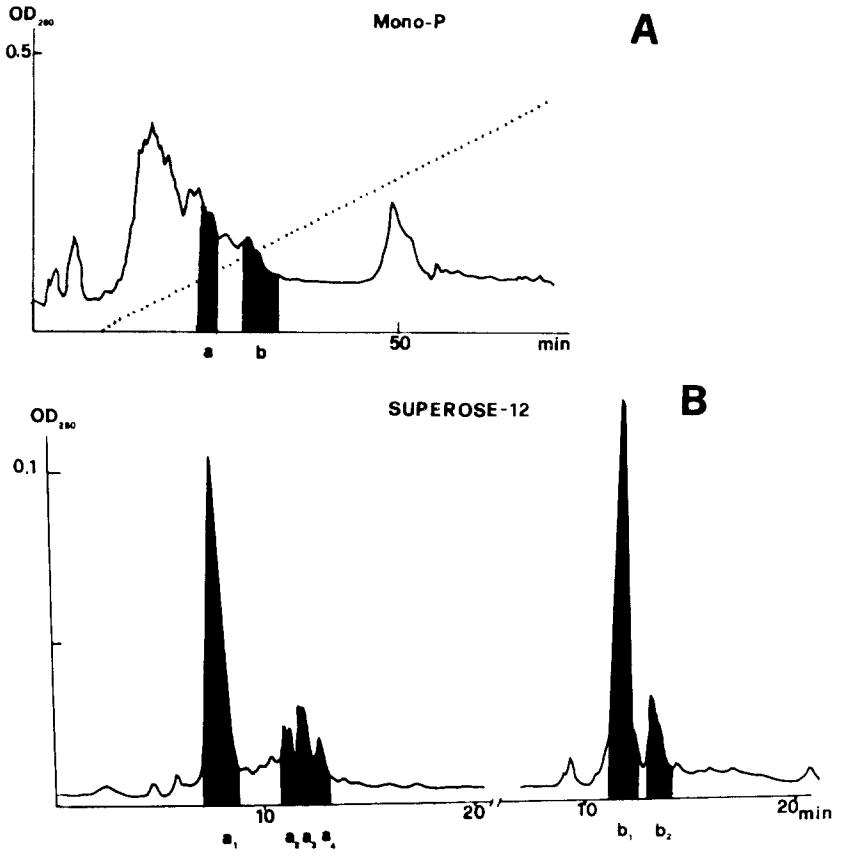

Fig. 4 Purification of synaptic redox enzymes by means of FPLC. Elution profiles of chromatography from extracts of synaptic plasma membranes partially purified by FPLC on Mono-P (A) followed by Superose-12 (Pharmacia) (B). The buffer systems for chromatofocusing on Mono-P consisted of 75 mM Tris-acetate, pH 9.3, which contained 0.5% Lubrol and 10% glycerol, and elution was performed with 10% Polybuffer 96 (Pharmacia) at pH 6.0 containing 0.5% Lubrol and 10% glycerol. The active fractions from the first column were immediately processed on Superose-12 equilibrated in 75 mM Tris-acetate, pH 7.4, containing 0.5% Lubrol, 10% glycerol, and 0.2 M NaCl.

classical chromatography by means of FPLC (Fig. 4). Solubilized extracts in 10–20% glycerol were applied on Mono-P (Pharmacia) columns equilibrated in 75 mM Tris-acetate, pH 9.3, containing 1.0% sodium deoxycholate and 0.5% Lubrol, and chromatofocusing was performed with 10% Ploybuffer 96 (Pharmacia), pH 6.0, containing 0.5% Lubrol. All buffers contained 10% glycerol in addition, a requisite to getting reproducible separations together with active enzyme preparations. Under these conditions, two peaks with NADH-dehydrogenase activity were resolved. Fraction a exhibited activity

with both ferricyanide and DCIP as cosubstrates in the NADH-dehydrogenase assay; in contrast, fraction b was only active with DCIP and displayed no activity with ferricyanide. Further purification of the active, pooled, and concentrated fractions was achieved by rechromatography on Superose-12 (Pharmacia) in 75 mM Tris-acetate, pH 7.55, containing 0.5% Lubrol and 0.2 M NaCl in 10% glycerol. Fraction a from the first column resolved into four active fractions by gel filtration under these experimental conditions, with one major peak with molecular mass of ca. 62.0 kDa (from SDS-polyacrylamide gel electrophoresis) and three minor active peaks of molecular masses between 19 and 30 kDa. Fraction a from the Mono-P column apparently corresponds to NADH-dehydrogenases IV obtained through band excision (from 2-D gel electrophoresis, data not shown). Fraction b from the Mono-P column was also subjected to the same chromatographic procedure and resolved into two active protein peaks. Activity on each peak was only detectable with DCIP and NADH substrates, not with ferricyanide. The major protein peak (peak b_1) displayed a molecular mass of ca. 65.0 kDa on SDS-polyacrylamide gel electrophoresis and a 2-D electrophoretic pattern similar to those from dehydrogenase V obtained through band excision.

NADH-acceptor oxidoreductases purified in this study display a complex heterogeneity. This situation recalls to mind the complexity of polypeptide composition in the mitochondrial respiratory chain complexes (Hatefi *et al.*, 1979). Mitochondrial complex I is composed of approximately 25 polypeptides (Hatefi, 1985), divided into three groups: the HP (hydrophobic fraction) made of very hydrophobic, small and highly alkaline polypeptides, the IP (water-soluble iron-protein fraction) with peptides of M_r of 75, 49, 30, 18, 15 and 13 kDa, and the FP (water-soluble flavoprotein fraction) which contains peptides with M_r of 51, 24, and 9 kDa. The greater simplicity of the 2-D pattern and especially the absence of peptides with low M_r (e.g., peptides with M_r between 33 and 8 kDa, corresponding to peptides 5–17 of complex I) in our preparations is therefore interesting. Similarly, mitochondrial complex II is composed of eight polypeptides (Capaldi *et al.*, 1977), including five peptides with low M_r, and complex III contains seven peptides of relatively low M_r (DasGupta and Rieske, 1973), in contrast to our observations on purified dehydrogenases from synaptic membranes. However, the presence of lipids in our proteins may also alter the electrophoretic pattern and therefore the estimates of M_r, explaining the relatively high molecular masses observed in this study. Further data on delipidated preparations are therefore required in order to improve our picture of synaptic redox enzymes.

The data presented in this paper reflect both the structural complexity of NADH-dehydrogenases in synaptic plasma membranes and the heterogeneity of the membrane preparations obtained routinely according to classical

procedures. The different NADH-dehydrogenases found by our methods may well be of diverse origin, pre- or post-synaptic as well as from glial plasma membranes. Evidence for plasma membrane dehydrogenases in cells of neuronal origin has been reported (Mersel *et al.*, 1984, 1988). It has been shown that plasma membranes from rat astrocytes in primary cultures displayed no NADH–cytochrome *c* reductase activity, but were endowed with a dehydrogenase system capable of catalyzing the transfer of reducing equivalents from NADH or NADPH to artificial acceptors such as DCIP or ferricyanide and exhibited DT-diaphorase-like activity (Mersel *et al.*, 1984). A role of the latter in cellular division or maturation has been proposed (Mersel *et al.*, 1988). It seems reasonable to assume that at least one of the bands described in this study might be attributable to these glial redox systems. The resolution of the diverse NADH-dehydrogenases in synaptic plasma membranes detailed in our study is a first step toward further studies aimed at a correct estimation of their function and specific localization in the brain membranes.

Acknowledgments

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